AF/1616

**Practitioner's Docket No. 16644/09005CIP** ***PATENT***

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re application of: Stephen Brian Falder, David Rawden

Application No.: 10/039,677
Filed: 01/04/2002
For: ANTI-MICROBIAL COMPOSITION

Group No.: 1616
Examiner: Alton N. Pryor

**Mail Stop Appeal Brief- Patents**
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1. Fee Transmittal (1 page)
2. Brief on Appeal w/Appendices A -C (60 pages)
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# FEE TRANSMITTAL For FY 2008

☐ Applicant claims small entity status. See 37 CFR 1.27

**TOTAL AMOUNT OF PAYMENT** ($) 510.00

| Complete if Known | |
|---|---|
| Application Number | 10/039,677 |
| Filing Date | January 4, 2002 |
| First Named Inventor | Falder, et al. |
| Examiner Name | Alton N. Pryor |
| Art Unit | 1616 |
| Attorney Docket No. | 16644/09005 |

**METHOD OF PAYMENT** (check all that apply)

☑ Check ☐ Credit Card ☐ Money Order ☐ None ☐ Other (please identify): _____

☑ Deposit Account Deposit Account Number: 50-2548 Deposit Account Name: Nelson Mullins Riley & Scarborough, LLP

For the above-identified deposit account, the Director is hereby authorized to: (check all that apply)

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☑ Charge any additional fee(s) or underpayments of fee(s) under 37 CFR 1.16 and 1.17 ☑ Credit any overpayments

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**FEE CALCULATION**

**1. BASIC FILING, SEARCH, AND EXAMINATION FEES**

| Application Type | FILING FEES Fee ($) | FILING FEES Small Entity Fee ($) | SEARCH FEES Fee ($) | SEARCH FEES Small Entity Fee ($) | EXAMINATION FEES Fee ($) | EXAMINATION FEES Small Entity Fee ($) | Fees Paid ($) |
|---|---|---|---|---|---|---|---|
| Utility | 310 | 155 | 510 | 255 | 210 | 105 | |
| Design | 210 | 105 | 100 | 50 | 130 | 65 | |
| Plant | 210 | 105 | 310 | 155 | 160 | 80 | |
| Reissue | 310 | 155 | 510 | 255 | 620 | 310 | |
| Provisional | 210 | 105 | 0 | 0 | 0 | 0 | |

**2. EXCESS CLAIM FEES**

| Fee Description | Fee ($) | Small Entity Fee ($) |
|---|---|---|
| Each claim over 20 (including Reissues) | 50 | 25 |
| Each independent claim over 3 (including Reissues) | 210 | 105 |
| Multiple dependent claims | 370 | 185 |

| Total Claims | Extra Claims | Fee ($) | Fee Paid ($) |
|---|---|---|---|
| _____ - 20 or HP = | _____ x | _____ = | N/A |

HP = highest number of total claims paid for, if greater than 20.

| Indep. Claims | Extra Claims | Fee ($) | Fee Paid ($) |
|---|---|---|---|
| _____ - 3 or HP = | _____ x | _____ = | N/A |

HP = highest number of independent claims paid for, if greater than 3.

**Multiple Dependent Claims**

| Fee ($) | Fee Paid ($) |
|---|---|
| | |

**3. APPLICATION SIZE FEE**

If the specification and drawings exceed 100 sheets of paper (excluding electronically filed sequence or computer listings under 37 CFR 1.52(e)), the application size fee due is $260 ($130 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).

| Total Sheets | Extra Sheets | Number of each additional 50 or fraction thereof | Fee ($) | Fee Paid ($) |
|---|---|---|---|---|
| _____ - 100 = | _____ / 50 = | _____ (round **up** to a whole number) x | _____ = | |

**4. OTHER FEE(S)**

| | Fees Paid ($) |
|---|---|
| Non-English Specification, $130 fee (no small entity discount) | |
| Other (e.g., late filing surcharge): Fees for filing Brief on Appeal | $510.00 |

**SUBMITTED BY**

| | | | |
|---|---|---|---|
| Signature | Nichole Andrighetti | Registration No. (Attorney/Agent) 56,508 | Telephone 864-250-2292 |
| Name (Print/Type) | Nichole T. Andrighetti | | Date 06/19/2008 |

**ATTORNEY DOCKET NO.: 16644/09005**

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | |
|---|---|---|
| Applicants: Falder, *et al.* | ) | Group Art Unit: 1616 |
| Serial No.: 10/039,677 | ) | Examiner: Alton N. Pryor |
| Filed: January 4, 2002 | ) | Deposit Account: 50-2548 |
| For: Anti-Microbial Composition | ) | |

## APPLICANT'S BRIEF ON APPEAL

Dear Sir or Madam:

Applicant submits the present Appeal Brief in accordance with 37 C.F.R. § 41.37. The Notice of Appeal was received by the USPTO on April 28, 2008, making the present Brief due on June 28, 2008. The Appeal Brief is being deposited as First Class Mail on the date noted on the attached Certificate of Mailing and is believed to be filed within the period for response. Please charge any additional fees that may be required to Deposit Account No. 50-2548.

06/23/2008 LTRUONG 00000036 10039677
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~~06/23/2008 LTRUONG 00000035 10039677~~
~~01 FC:1402 510.00 OP~~

Void date: 06/23/2008 LTRUONG
06/23/2008 LTRUONG 00000035 10039677
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## 1. Real Party In Interest

The real party in interest with respect to the above-captioned application and with respect to this Appeal is the assignee of the above-captioned application, Byotrol, PLC of Riverside Works, Collyhurst Rd., Manchester, Greater Manchester, M2 2JF, United Kingdom.

## 2. Related Appeals and Interferences

There are no related appeals, interferences or judicial proceedings known to Applicant, Applicant's legal representative, or any assignee that will directly affect, be directly affected by or have a bearing on the Board's decision in the present appeal. The "Related Proceedings Appendix" indicates that there are no related appeals or interferences.

## 3. Status of the Claims

The present application was filed on January 4, 2002, as a continuation-in-part application, claiming the priority benefit of U.S. Patent Application Serial No. 09/756,457, filed on January 8, 2001. The application lists Stephen Brian Falder and David Rawden as inventors.

Claims 1, 46, 52, 53, 61, 62, 70, 71, 78, 82-85, 88-92, 95-100, 105-107, 111-113, and 115-137 are currently pending, have been rejected, and are currently being appealed. Claims 1, 106, and 137 are independent claims. A copy of the presently pending claims is attached hereto as the "Claims Appendix".

## 4. Status of Amendments

No amendments were filed subsequent to the January 24, 2008, Final Office Action.

## 5. Summary of Claimed Subject Matter

Broadly speaking, the invention of the above-captioned application is directed to anti-microbial compositions and formulations. Pg. 7, lines 10-11, 17-18. As noted, claims 1, 106, and 137 are independent claims. Claim 1 of the application reads as follows:

Claim 1

An anti-microbial composition consisting essentially of:

(i) at least one anti-microbial agent, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m, and is selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups, (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl groups are selected from alkyl groups comprising from 8 to 12 carbon atoms, and (c) a benzalknoium halide or an aryl ring substituted benzalkonium halide,

(ii) at least one compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, polydimethylhydroxysiloxanes, and mixtures thereof, and

(iii) at least one polar solvent, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied.

The elements of claim 1 can be located in the specification as follows. The anti-microbial composition consists essentially of at least one anti-microbial agent, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m. Pg. 7, lines 10-15; pg. 10, lines 6-7 and 28. The at least one anti-microbial agent of claim 1 is selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen

and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups (pg. 12, lines 8-20), (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl groups are selected from alkyl groups comprising from 8 to 12 carbon atoms (pg. 12, lines 25-27), and (c) a benzalknoium halide or an aryl ring substituted benzalkonium halide (pg. 12, lines 22-23).

The anti-microbial composition of the invention also consists essentially of at least one compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, polydimethylhydroxysiloxanes, and mixtures thereof. Pg. 7, lines 12-13; pg. 10, lines 9-10; pg 15, lines 16-21.

The anti-microbial composition of the invention additionally consists essentially of at least one polar solvent, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied. Pg. 7, lines 13-15; pg. 8, lines 1-4, 23-25. Reference to the drawings would be inapplicable to explain the subject matter defined by claim 1.

Claim 106

> An anti-microbial composition containing as a solvent a polar solvent which is selected from the group consisting of water, at least one alcohol, at least one glycol ester, at least one polyol, at least one ketone or a mixture thereof, and comprising:
>
> (i) at least one anti-microbial agent, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m and selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups, (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl

groups are selected from medium and long chain alkyl groups comprising from 8 to 12 carbon atoms, and (c) a benzalkonium halide or an aryl ring substituted benzalkonium halide; and

(ii) at least one compound having a low surface tension of from 8 to 14 mN/m and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, polydimethylhydroxysiloxanes, and mixtures thereof, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied.

The elements of claim 106 can be located in the specification as follows. The anti-microbial composition contains a solvent a polar solvent which is selected from the group consisting of water, at least one alcohol, at least one glycol ester, at least one polyol, at least one ketone or a mixture thereof. Pg. 7, lines 13-15; pg. 15, lines 29-30.

The anti-microbial composition also comprises at least one anti-microbial agent, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m. Pg. 7, lines 10-15; pg. 10, lines 6-7 and 28. The at least one anti-microbial agent of claim 1 is selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups (pg. 12, lines 8-20), (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl groups are selected from medium and long chain alkyl groups comprising from 8 to 12 carbon atoms (pg. 12, lines 25-27), and (c) a benzalkonium halide or an aryl ring substituted benzalkonium halide (pg. 12, lines 22-23).

The anti-microbial composition of the invention additionally comprises at least one compound having a low surface tension of from 8 to 14 mN/m and selected from

the group consisting of silanes, soya lecithins, polydimethylsiloxanes, polydimethylhydroxysiloxanes, and mixtures thereof (pg. 7, lines 12-13; pg. 10, lines 9-10; pg 15, lines 16-21), wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied (Pg. 8, lines 1-4, 23-25). Reference to the drawings would be inapplicable to explain the subject matter defined by claim 106.

Claim 137

An anti-microbial composition consisting essentially of:

(i) at least two anti-microbial agents, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m, and is selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups, (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl groups are selected from alkyl groups comprising from 8 to 12 carbon atoms, and (c) a benzalknoium halide or an aryl ring substituted benzalkonium halide,

(ii) a compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, and polydimethylhydroxysiloxanes, and

(iii) a polar solvent, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied.

The elements of claim 137 can be located in the specification as follows. The anti-microbial composition consists essentially of at least two anti-microbial agents, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m. Pg. 7, lines 10-15; pg. 10, lines 6-7 and 28. The at least one anti-microbial agent of claim 1 is selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in

which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups (pg. 12, lines 8-20), (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl groups are selected from alkyl groups comprising from 8 to 12 carbon atoms (pg. 12, lines 25-27), and (c) a benzalknoium halide or an aryl ring substituted benzalkonium halide (pg. 12, lines 22-23).

The anti-microbial composition also consists essentially of a compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, and polydimethylhydroxysiloxanes (pg. 7, lines 12-13; pg. 10, lines 9-10; pg 15, lines 16-21). Additionally, the anti-microbial composition consists essentially of a polar solvent, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied. Pg. 7, lines 13-15; pg. 8, lines 1-4, 23-25. Reference to the drawings would be inapplicable to explain the subject matter defined by claim 137.

**6. Grounds of Rejection to be Reviewed on Appeal**

Only one prior art rejection remains at issue. Claims 1, 46, 52, 53, 61, 62, 70, 71, 78, 82-85, 88-92, 95-100, 105-107, 111-113, and 115-137 were rejected under 35 U.S.C. § 103(a) as unpatentable over Trinh (U.S. Patent No. 6,656,923). The ground of rejection to be reviewed is whether the claimed invention is unpatentably obvious over Trinh.

## 7. Arguments

In the Final Office Action, the pending claims were rejected under 35 U.S.C. § 103(a) over Trinh. However, it is respectfully submitted that Trinh fails to teach or suggest all of the claim limitations. Namely, Trinh fails to teach or suggest "a compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, and polydimethylhydroxysiloxanes," present in each of the independent claims. Because Trinh does not teach or suggest all of the claim limitations, the Examiner has not established a *prima facie* case of obviousness.

### a. The Examiner Has Failed to Establish a *Prima Facie* Case of Obviousness Because Trinh Does Not Teach or Suggest All of the Claim Elements.

In determining whether a claim is obvious, the Examiner must make "a searching comparison of the claimed invention – *including all its limitations* – with the teaching of the prior art." *In re Ochiai*, 71 F.3d 1565, 1572 (Fed. Cir. 1995) (emphasis added). Thus, "obviousness requires a suggestion of all limitations in a claim." *CFMT, Inc. v. Yieldup Intern. Corp.*, 349 F.3d 1333, 1342 (Fed. Cir. 2003) (*citing In re Royka*, 490 F.2d 981, 985 (CCPA 1974)). In the present case, the Examiner has failed to establish a *prima facie* case of obviousness because all of the claim limitations are not taught or suggested by Trinh.

Specifically, Trinh fails to teach "a compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, and polydimethylhydroxysiloxanes." The Examiner asserts that the composition of Trinh "can comprise dimethyl polysiloxanes," citing Trinh, col. 9, lines

47 – col. 13, line 38, but especially col. 11, line 48 – col. 12, line 63. January 24, 2008 Office Action, page 5. While the terms "dimethyl polysiloxane" and "polydimethylsiloxane" are synonymous, *Merck Index* 3260 (Susan Dudavari, ed., 12th ed., Merck & Co., Inc. 1996), the Examiner is simply mistaken in his assertion that the composition of Trinh "can comprise dimethyl polysiloxanes." Trinh actually teaches the use of "polyalkylene oxide polysiloxanes having a dimethyl polysiloxane hydrophobic moiety and one or more hydrophilic polyalkylene side chains." Trinh, col. 11, lines 48 – 51 (emphasis added). As explained herein, polyalkylene oxide polysiloxanes are not polydimethylsiloxanes. While Trinh teaches a compound having a dimethyl polysiloxane moiety, it does not, as asserted by the Examiner, teach or suggest the use of polydimethylsiloxanes.

**i. Polyalkylene Oxide Polysiloxanes are Not Structurally Similar to the Polydimethylsiloxanes of the Present Invention.**

Polydimethylsiloxanes, as is understood among those of ordinary skill in the art, have the following formula:

$(CH_3)_3$-Si-O-$(Si(CH_3)_2$-O$)_n$-Si$(CH_3)_3$

*Merck Index* at 3260.

In contrast, the polyalkylene oxide polysiloxanes of Trinh, as specifically described in Trinh, have the formula:

$R^1$-$(CH_3)_2$SiO-$[(CH_3)_2SiO]_a$-$[(CH_3)(R^1)SiO]_b$-Si$(CH_3)_2$-$R^1$

> wherein a+b are from about 1 to about 50, preferably from about 3 to about 30, more preferably from about 10 to about 25, and each $R^1$ is the same or different and is selected from the group consisting of methyl and a poly(ethyleneoxide/propyleneoxide) copolymer group having the general formula:
>
> -$(CH_2)_n O(C_2H_4O)_c(C_3H_6O)_d R^2$

> with at least one $R^1$ being a poly(ethyleneoxide/propyleneoxide) copolymer group, and wherein n is 3 or 4, preferably 3; total c (for all polyalkyleneoxy side groups) has a value of from 1 to about 100, preferably from about 6 to about 100; total d is from 0 to about 14, preferably from 0 to about 3; and more preferably d is 0; total c+d has a value of from about 5 to about 150, preferably from about 9 to about 100 and each $R^2$ is the same or different and is selected from the group consisting of hydrogen, an alkyl having 1 to 4 carbon atoms, and an acetyl group, preferably hydrogen and methyl group. Each polyalkylene oxide polysiloxane has at least one $R^1$ group being a poly(ethyleneoxide/propyleneoxide) copolymer group.
>
> Trinh, col. 11, lines 48 – col. 12, line 10.

As illustrated above, the surfactant taught in Trinh is substantially different in structure from the polydimethylsiloxanes of the present invention. Polyalkylene oxide polysiloxanes merely have a dimethyl polysiloxane moiety as a component thereof. One of ordinary skill in the art understands that the term "moiety is generally used to signify part of a molecule." IUPAC Compendium of Chemical Terminology (2d ed. 1997) (emphasis added). Thus, the Trinh surfactant contains dimethyl polysiloxane as only part of the total molecule. The polyalkylene oxide polysiloxanes of Trinh additionally contain various hydrophilic polyalkylene side chains, indicated as $R^1$ moieties. As set forth in Trinh, $R^1$ is "the same or different and is selected from the group consisting of methyl and a poly(ethyleneoxide/propyleneoxide) copolymer group having the general formula:

$-(CH_2)_nO(C_2H_4O)_c(C_3H_6O)_dR^2$," separately defining n, c, d, and $R^2$. Trinh, col. 11, lines 58-63. Based upon the many options for $R^1$, there are *innumerable* possibilities of compounds, none of which is polydimethylsiloxane. In addition, none of the possibilities is even structurally similar to polydimethylsiloxane. Trinh requires that "[e]ach polyalkylene oxide polysiloxane has at least one $R^1$ group being a

poly(ethyleneoxide/propyleneoxide) copolymer." Trinh, col. 12, lines 8-10. Even if the other two $R^1$ groups were methyl groups, which in itself would create a structurally different compound, the mandatory poly(ethyleneoxide/propyleneoxide) copolymer group creates a molecular structure which is substantially different from that of polydimethylsiloxane. Because the molecules are not structurally similar, Trinh does not teach or suggest every limitation of the present claims and cannot render the present claims obvious.

**ii. Polyalkylene Oxide Polysiloxanes Have Significantly Different Physical Properties Than the Polydimethylsiloxanes of the Present Invention.**

In addition to their structural differences, the polyalkylene oxide polysiloxanes of Trinh also have significantly different physical properties than the polydimethylsiloxanes used in the present invention. For example, the polyalkylene oxide polysiloxanes of Trinh, again as specifically described by Trinh itself, comprise both hydrophobic and hydrophilic moieties and must be water dispersible or water soluble: "the number of ethyleneoxy units ($-C_2H_4O$) in the polyether chain (RI) must be sufficient to render the polyalkylene oxide polysiloxane water dispersible or water soluble." Trinh, col. 12, lines 36-39. In contrast, the polydimethylsiloxanes of the present invention are entirely hydrophobic and are not water dispersible or water soluble. *See Merck Index* at 3260 (Dimethylpolysiloxane is "immiscible with water."). The hydrophobic nature of the polydimethylsiloxane used in the present invention is extremely important for providing the unique anti-microbial properties exhibited by the composition. As Applicant has previously illustrated in the Declaration of Dr. Ulrich Schwartz and Annexes I and II (submitted herewith in the "Evidence Appendix"), the claimed compositions have

advantageous and unexpected anti-microbial properties, particularly in terms of residual effect. This residual effect could not be achieved if the hydrophobic polydimethylsiloxane were replaced by a water-soluble or water-dispersible component that has both hydrophilic and hydrophobic moieties.

In the compositions of the present invention, there is an interaction between the anti-microbial agent(s) and the low surface tension material. As an example, when polydimethylsiloxane is the low surface tension material, it holds the anti-microbial agent(s) on the surface to which the composition is applied even if that surface is subsequently wetted or washed. If the hydrophobic polydimethylsiloxane were replaced by a water-soluble or water-dispersible material having both hydrophilic and hydrophobic moieties, this residual effect simply would not be achieved because the polyalkylene oxide polysiloxane would not interact with the anti-microbial agent to retain the anti-microbial agent on the surface. Instead, washing the surface would effectively remove the polyalkylene oxide polysiloxane and the anti-microbial agent from the surface. Such compositions would not exhibit the residual anti-microbial effect exhibited by the compositions of the invention. Thus, substitution of the polyalkylene oxide polysiloxane of Trinh for the polydimethylsiloxane of the invention would detrimentally affect the properties of the anti-microbial composition.

In addition, at column 12, lines 12 to 15, Trinh states that Silwet® surfactants are examples of the polyalkylene oxide polysiloxanes. Silwet® surfactants are well known among those of skill in the art. For example, U.S. Patent No. 5,543,048, which issued and published on August 6, 1996, before Trinh was filed, discusses the properties of Silwet® surfactants:

> Silwet® surfactants are chemically unique and should not be confused with conventional polydimethylsiloxanes because they are composed of a siloxane backbone with organic polyalkylene oxide pendants, forming chemical structures whose variations provide a wide range of useful performance characteristics.
>
> U.S. Patent No. 5,543,048, col. 3, lines 31-36 (emphasis added).

This reference makes clear that it is well known in the art that polyalkylene oxide polysiloxanes and polydimethylsiloxanes are very different compositions, both structurally and physically, that should not be confused with one another. Because of their structural and physical differences, the compounds cannot be obvious variations of one another. Thus, Trinh fails to teach or suggest the use of polydimethylsiloxane or any other low surface tension material, leaving the reference insufficient to establish a *prima facie* case of obviousness.

**b. Even If a *Prima Facie* Case of Obviousness Has Been Satisfied, Applicant Has Provided Substantial Evidence of Unexpected Results, Overcoming Any *Prima Facie* Case.**

Applicant has previously submitted the Declaration of Dr. Ulrich Schwartz and Annexes I and II, illustrating the unexpected and surprising results of the inventive composition. Significantly, the experiments summarized by Dr. Schwartz in this Declaration and further detailed in Annexes I and II indicate that (i) the compositions of the invention have improved anti-microbial properties compared to identical compositions that do not contain a low surface tension material, as such is claimed in the application; and (ii) the compositions of the invention have a residual anti-microbial effect. A person of ordinary skill in the art, upon reading Trinh, could not possibly have predicted that a composition as claimed could provide these advantageous properties.

As discussed in paragraph 10 of the Declaration and described in further detail in Annex I, an experiment was conducted to illustrate the residual anti-microbial effect of compositions of the invention. In this experiment, the composition of the present invention was applied to a bathroom surface in two passenger cabins in a cruiser liner. The surface was then cleaned on a daily basis using water only. The total microbial count on the surface was tested on the third and the seventh day after application of the composition. The results indicate that the compositions of the invention provided a residual anti-microbial effect, largely reducing or controlling the formation of colonies of microorganisms on a surface for up to a week (the limit of this experiment) after application of the composition, even when that surface is washed daily with water.

Some further experiments to illustrate the residual anti-microbial effect of the compositions of the invention are described in Annex II and discussed in paragraph 11 of the Declaration. A composition of the invention was applied to a surface and allowed to dry on that surface. When the surface was dry, a protein solution (representing a microbial biofilm) was applied to the surface and was dried. The surface was then rinsed extensively with distilled water. The water rinsing removed most of the protein solution from the surface, but did not remove the inventive composition from the surface. A comparative test was also carried out in which the same procedure was followed except that an anti-microbial composition that did not comprise a low surface tension compound was used in place of the composition of the invention. In this comparative test, rinsing extensively with distilled water did not remove the protein layer. These results indicate that a microbial biofilm would be unable to adhere to a surface treated with the composition of the present invention while it would be able to

adhere to a surface treated with the comparative anti-microbial composition having no low surface tension composition. This experiment illustrates the residual anti-microbial effect of the inventive composition in that in addition to killing microbes present at a surface at the time a surface is treated with the composition, further microbial biofilm formation at that surface is also prevented.

This residual anti-microbial effect was particularly surprising in light of the prior art failings to provide an anti-microbial composition which retains its effectiveness over an extended time period. An example of such failure is described in Applicant's specification, "bleaches do not provide long-term, passive anti-microbial control and sanitisation. By "passive control" we mean that the substrate counters microbial infection on its own by some property within it, so that it does not require a cleaning regime to be effective at controlling microorganisms." Falder, pg. 4, lines 20-23. In addition, "some anti-microbial agents, such as biphenol, do not remain active for extended periods." Falder, pg. 5, lines 24-25. Thus, the residual anti-microbial effect of the present invention was a surprising and unexpected result, representing an advancement in the anti-microbial arts.

As discussed in paragraphs 12 to 15 of the Declaration, Annex I also reports a number of experiments that were conducted to illustrate the surprising and unexpected anti-microbial effect that is achieved by the combination of a quaternary ammonium compound and a low surface tension material, as defined in the claims of the present application, as compared to a quaternary ammonium compound in the absence of a low surface tension material.

For example, in one of the experiments described in Annex I, cotton cloths were soaked in three compositions or deionized water (control). The three compositions were a solution of low surface tension material such as Clearco (a polysiloxane), a solution of a quaternary ammonium compound and a solution of Clearco and the quaternary ammonium compound. The treated cloths were stored at room temperature until they were completely dry. Each of the dry pre-treated cloths and the control cloths were placed in Petri dishes filled with 5 mL of deionised water and left to stand for 5 minutes. The washed cloths were then dried. Each dry, washed cloth was challenged with 300 µL E. Coli and allowed to completely dry at room temperature. When dry, the cloths were incubated at 37°C for 2 hours. Deionised water was then added to each Petri dish to elute surviving E. coli. The plates were evaluated for viable colony forming units.

In the experiments conducted, the quaternary ammonium compounds were effective only at concentrations of 1.5% and 0.07%, but not at 0.025%. However, when a low surface tension material was used in combination with a quaternary ammonium compound, the anti-microbial properties of the resulting composition were unexpectedly enhanced. In fact, when the low surface tension material was used in combination with the quaternary ammonium compound as claimed in the present invention, the composition was effective as an anti-microbial agent at concentrations of 2.5%, 1.5%, 0.125%, 0.07%, 0.042%, and even 0.025%. These results were confirmed in a second experiment, which tested the compositions over a broader concentration range.

In summary, these experiments clearly show that the composition of the present invention is unexpectedly more effective as an anti-bacterial agent than a composition

which does not contain a low surface tension material. This result was especially surprising because the low surface tension material does not have any anti-microbial effect itself, yet it significantly enhanced the anti-microbial effect of the inventive compound.

In conclusion, the experimental results reported in the Declaration and its Annexes illustrate both the surprising and unexpected residual anti-microbial effects of the compositions of the invention and the surprising and unexpected enhanced anti-microbial effect that is achieved by the combination of an anti-microbial composition, as claimed, and a low surface tension material, as claimed, in a polar solvent. The skilled person could not have predicted that the claimed composition would have this combination of advantageous properties on the basis of merely reading Trinh.

Finally, the Examiner noted on page 6 of the Final Office Action that "the simple act of combining and mixing ingredients is common in this art therefore the method of making the composition as described in claim 99 is not patentable." Applicant points out that claim 99 is actually dependent on claim 1. Thus, if claim 1 is allowable, which Applicant believes it is, claim 99 should also be allowable. Additionally, the Examiner has not provided any evidence that combining and mixing these specific ingredients in this specific manner is known in the art. Thus, the Examiner has not established a *prima facie* case that claim 99 of the application is obvious.

In summary, the present invention claims various anti-microbial compositions and formulations. Trinh simply does not teach all of the elements of the claimed anti-microbial composition. Namely, Trinh does not teach the inclusion of a low surface tension material, as claimed in each of the independent claims. For at least this reason,

the Examiner has not established a *prima facie* case of obviousness. Even if this Board should consider the *prima facie* case to have been established, Applicant has provided substantial evidence showing the surprising and unexpected benefits of the present invention, indicating that the invention would not, in fact, be considered obvious in light of Trinh. Thus, it is respectfully requested that Applicant's Appeal be granted and a patent issue based on the above-captioned application.

Please charge any additional fees required by this Appeal Brief to Deposit Account No. 50-2548.

Respectfully requested,
NELSON MULLINS RILEY & SCARBOROUGH

6-19-08
Date

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A



## Claims Appendix

1. An anti-microbial composition consisting essentially of:

(i) at least one anti-microbial agent, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m, and is selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups, (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl groups are selected from alkyl groups comprising from 8 to 12 carbon atoms, and (c) a benzalknoium halide or an aryl ring substituted benzalkonium halide,

(ii) at least one compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, polydimethylhydroxysiloxanes, and mixtures thereof, and

(iii) at least one polar solvent, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied.

2. – 45. (canceled).

46. An anti-microbial composition according to Claim 1, wherein the surface tension of the at least one compound (ii) is 10 mN/m.

47 – 51. (canceled).

52. An anti-microbial composition according to Claim 1, wherein at least one of the anti-microbial agents is of a polar nature.

53. An anti-microbial composition according to Claim 1, comprising at least one anti-microbial agent selected from bacteriocidal, fungicidal, algicidal, yeasticidal and moldicidal agents.

54. – 60. (canceled).

61. An anti-microbial composition according to Claim 1, wherein at least one of the anti-microbial agents is selected from benzenemethanaminium N-dodecyl-N,N-dimethylchloride, and benzyl-$C_{12}$-$C_{16}$-alkyldimethyl-ammoniumchloride.

62. An anti-microbial composition according to Claim 1, wherein at least one of the anti-microbial agents is selected from an amphoteric compound, an iodophore, a phenolic compound, a quaternary ammonium compound, a hypochlorite and a nitrogen based heterocyclic compound.

63. – 69. (canceled).

70. An anti-microbial composition according to Claim 62, wherein the or each phenolic compound is selected from a methyl, ethyl, butyl, halo and aryl substituted phenol.

71. An anti-microbial composition according to Claim 62, wherein the or each phenolic compound is selected from 2-phenylphenol, 2-benzyl-4-chlorophenol, 2-cyclopentanol-4-chlorophenol, 4-t-amylphenol, 4-t-butylphenol, 4-chloro-2-pentylphenol, 6-chloro-2-pentylphenol, p-chlorometa-xylenol, 2,4,4-trichloro-2-hydroxydiphenol, thymol, 2-i-propyl-3-methylphenol, chlorothymol, 3-methyl-4-chlorophenol, 2,6-dichloro-4-n-alkyl phenols, 2,4-dichloro-meta-xylenol, 2,4,6-trichlorophenol and 2-benzyl-4-chlorophenol.

72. – 77. (canceled).

78. A composition according to Claim 1, wherein at least one of the anti-microbial agents is selected from benzenemethanaminium N-dodecyl-N,N-dimethylchloride, and benzyl-$C_{12}$-$C_{16}$-alkyldimethyl-ammoniumchloride, and at least one additional anti-microbial agent is selected from 2-phenylphenol, 2-octyl-2H-isothiazol-3-one, 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one.

79. – 81. (canceled).

82. An anti-microbial composition according to Claim 1, comprising from 1 to 4% by volume of the at least one compound (ii).

83. An anti-microbial composition according to Claim 1, wherein the at least one polar solvent is selected from water, an alcohol, an ester, a hydroxyl or glycol ester, a polyol, a ketone, and mixtures thereof.

84. An anti-microbial composition according to Claim 1, wherein the at least one polar solvent is selected from n-propanol, water, isopropanol, diethylene glycol and dipropylene glycol.

85. An anti-microbial composition according to Claim 1, comprising from 1 to 70% by volume of the at least one polar solvent.

86. – 87. (canceled)

88. A formulation comprising the anti-microbial composition according to Claim 1, and a functional material.

89. A formulation according to Claim 88, wherein the functional material is selected from plastics, fibres, coatings, films, laminates, adhesives, sealants,

clays, china, ceramics, concrete, sand, paints, varnishes, lacquers, cleaning agents and settable or curable compositions such as fillers, grouts, mastics and putties.

90. A formulation according to Claim 88, wherein the formulation comprises from 0.1 to 5.0% by weight of the anti-microbial composition.

91. A formulation according to Claim 88, wherein the formulation comprises from 0.5 to 2.0% by weight of the anti-microbial composition.

92. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the anti-microbial composition according to Claim 1 to the surface.

93. – 94. (canceled)

95. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 88 to the surface.

96. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 89 to the surface.

97. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 90 to the surface.

98. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 91 to the surface.

99. A method of manufacturing an anti-microbial composition according to Claim 1, the method comprising the steps of (a) mixing the anti-microbial agent and any additional anti-microbial agents together, (b) adding the at least one compound (ii) to the anti-microbial agent(s), (c) adding the at least one polar solvent to the mixture of the at least one compound (ii) and anti-microbial agent(s) and (d) agitating the resulting mixture until a clear solution is formed.

100. A method of manufacturing a formulation comprising the step of adding the anti-microbial composition of Claim 1 to a functional material.

101. – 104. (canceled).

105. An anti-microbial composition according to Claim 1, wherein the at least one compound (ii) is selected from polydimethylsiloxanes, and polydimethylhydrosiloxanes and mixtures thereof.

106. An anti-microbial composition containing as a solvent a polar solvent which is selected from the group consisting of water, at least one alcohol, at least one glycol ester, at least one polyol, at least one ketone or a mixture thereof, and comprising:

(i) at least one anti-microbial agent, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m and selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups, (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl

groups are selected from medium and long chain alkyl groups comprising from 8 to 12 carbon atoms, and (c) a benzalkonium halide or an aryl ring substituted benzalkonium halide; and

(ii) at least one compound having a low surface tension of from 8 to 14 mN/m and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, polydimethylhydroxysiloxanes, and mixtures thereof, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied.

107. An anti-microbial composition according to Claim 106, wherein the surface tension of the at least one compound (ii) is 10 mN/m.

108. - 110. (canceled).

111. An anti-microbial composition according to Claim 106 comprising at least one additional anti-microbial agent.

112. An anti-microbial composition according to Claim 111, wherein at least one of the anti-microbial agents is of a polar nature.

113. An anti-microbial composition according to Claim 106 comprising at least one anti-microbial agent selected from bacteriocidal, fungicidal, algicidal, yeasticidal and moldicidal agents.

114. (canceled).

115. An anti-microbial composition according to Claim 106, wherein at least one of the anti-microbial agents is selected from benzenemethanaminium N-dodecyl-N,N-dimethylchloride, and benzyl-$C_{12}$-$C_{16}$-alkyldimethyl-ammoniumchloride.

116. An anti-microbial composition according to Claim 111, wherein the at least one additional anti-microbial agent is selected from amphoteric compounds, iodophores, phenolic compounds, quarternary ammonium compounds, hypochlorites and nitrogen-based heterocyclic compounds.

117. An anti-microbial composition according to Claim 116, wherein the or each phenolic compound is selected from a methyl, ethyl, butyl, halo and aryl substituted phenol.

118. An anti-microbial composition according to Claim 116, wherein the or each phenolic compound is selected from 2-phenylphenol, 2-benzyl-4-chlorophenol, 2-cyclopentanol-4-chlorophenol, 4-t-amylphenol, 4-t-butylphenol, 4-chloro-2-pentylphenol, 6-chloro-2-pentylphenol, p-chlorometa-xylenol, 2,4,4-trichloro-2-hydroxydiphenol, thymol, 2-i-propyl-3-methylphenol, chlorothymol, 3-methyl-4-chlorophenol, 2,6-dichloro-4-n-alkyl phenols, 2,4-dichloro-meta-xylenol, 2,4,6-trichlorophenol and 2-benzyl-4-chlorophenol.

119. A composition according to Claim 111, wherein at least one of the anti-microbial agents is selected from benzenemethanaminium N-dodecyl-N,N-dimethylchloride and benzyl-$C_{12}$-$C_{16}$-alkyldimethyl-ammoniumchloride, and at least one of the additional anti-microbial agents is selected from 2-phenylphenol, 2-octyl-2H-isothiazol-3-one, 5-chloro-2-methyl-2H-isothiazol-3-one, and 2-methyl-2H-isothiazol-3-one.

120. An anti-microbial composition according to Claim 106, comprising from 1 to 4% by volume of the at least one compound (ii).

121. An anti-microbial composition according to Claim 106, wherein the polar solvent is selected from n-propanol, water, isopropanol, diethylene glycol, dipropylene glycol and mixtures thereof.

122. An anti-microbial composition according to Claim 106, comprising from 1 to 70% by volume of the polar solvent.

123. An anti-microbial composition according to Claim 106, wherein the at least one compound (ii) is selected from polydimethylsiloxanes, polydimethylhydrosiloxanes and mixtures thereof.

124. A formulation comprising the anti-microbial composition according to Claim 106, and a functional material.

125. A formulation according to Claim 124, wherein the functional material is selected from plastics, fibres, coatings, films, laminates, adhesives, sealants, clays, china, ceramics, concrete, sand, paints, varnishes, lacquers, cleaning agents and settable or curable compositions such as fillers, grouts, mastics and putties.

126. A formulation according to Claim 124, wherein the formulation comprises from 0.1 to 5.0% by weight of the anti-microbial composition.

127. A formulation according to Claim 124, wherein the formulation comprises from 0.5 to 2.0% by weight of the anti-microbial composition.

128. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the anti-microbial composition according to Claim 106 to the surface.

129. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 124 to the surface.

130. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 125 to the surface.

131. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 126 to the surface.

132. A method of reducing or controlling the formulation of colonies of microorganisms on a surface, which method comprises applying the formulation of Claim 127 to the surface.

133. A method of manufacturing an anti-microbial composition according to Claim 106, the method comprising the steps of (a) mixing the or each anti-microbial agents together, (b) adding the at least one compound (ii) to the mixture of step (a), (c) adding the polar solvent to the mixture of step (b), and (d) agitating the resulting mixture until a clear solution is formed.

134. A method of manufacturing a formulation comprising the step of adding the anti-microbial composition of Claim 106 to a functional material.

135. An antimicrobial composition according to claim 105, wherein the compound (ii) is selected from the group consisting of polydimethylsiloxane having a chain length of from $C_{12}$ to $C_{20}$ and polydimethylhydrosiloxane having a chain length of from $C_{12}$ to $C_{20}$.

136. An antimicrobial composition according to claim 123, wherein the compound (ii) is selected from the group consisting of polydimethylsiloxane having a chain length of from $C_{12}$ to $C_{20}$ and polydimethylhydrosiloxane having a chain length of from $C_{12}$ to $C_{20}$.

137. An anti-microbial composition consisting essentially of:

(i) at least two anti-microbial agents, wherein at least one of the anti-microbial agents is an anti-microbial agent having a high surface tension of from 20 to 35 mN/m, and is selected from the group consisting of (a) a quarternary ammonium compound having the general formula $R^1R^2R^3R^4N^+X^-$, in which one or two of the R groups are alkyl substituted by aryl or interrupted by aryl or oxygen and the other R groups are the same or different and are $C_1$ to $C_4$ alkyl groups, (b) a dialkyldimethylammonium compound wherein the two non-methyl alkyl groups are selected from alkyl groups comprising from 8 to 12 carbon atoms, and (c) a benzalknoium halide or an aryl ring substituted benzalkonium halide,

(ii) a compound having a low surface tension of from 8 to 14 mN/m, and selected from the group consisting of silanes, soya lecithins, polydimethylsiloxanes, and polydimethylhydroxysiloxanes, and

(iii) a polar solvent, wherein in use the anti-microbial composition acts substantially to reduce or control the formation of microbial colonies on or at a surface to which the composition is applied.

B

## Evidence Appendix

1. Declaration of Dr. Ulrich Swartz, submitted on January 12, 2007, under 37 C.F.R. § 1.132 as part of Applicant's response to the July 12, 2006 Office Action. The Examiner entered the Declaration into the record in the April 20, 2007 Office Action, pg. 2.

2. Annex I to Declaration of Dr. Swartz, submitted on January 12, 2007, under 37 C.F.R. § 1.132 as part of Applicant's response to the July 12, 2006 Office Action. The Examiner entered Annex I into the record in the April 20, 2007 Office Action, pg. 2.

3. Annex II to Declaration of Dr. Swartz, submitted on January 12, 2007, under 37 C.F.R. § 1.132 as part of Applicant's response to the July 12, 2006 Office Action. The Examiner entered Annex II into the record in the April 20, 2007 Office Action, pg. 2.

(1)

I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450, ON THE DATE INDICATED BELOW.

By: DEBRA DUNN-BROWN Date: 1/12/07



**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In Re Patent Application of:
Stephen Brian Falder *et al.*

Group Art Unit: 1616

Appln. No.: 10/039,677

Examiner: Alton N. Pryor

Filing Date: January 4, 2002

Attorney Docket No.: 16644/09005CIP
(BYOCX/P25765US)

Title: ANTI-MICROBIAL COMPOSITION

**DECLARATION OF UNDER 37 C.F.R. § 1.132**

I, Ulrich W Schwarz, declare as follows:

1. I am a German citizen residing in Germany. I have previously been engaged by Byotrol PLC as a consultant in relation to the subject matter of the present application and I am now an employee of Byotrol PLC. I understand that Byotrol PLC is the assignee of the above-identified application.

2. My professional experience includes Head of Clinical Lab (1979 – 1981), Medical Director Pharmaceutical Company (1982 – 1984), Scientific Adviser Drägerwerk AG in Industrial & Environmental Hygiene (1985 – 1992) (This special working area included microbial testing indoors (mould testing), testing of hygienic conditions in hospitals, food industry and other workplaces.), Consultant in Food & Environmental Hygiene (1992 – 1996) (This included microbial testing in food industry, surface testing, raw material testing and personal testing), Technical Director Medical Company developing *in vitro* Diagnostics (Sandwich Immuno Assay: hormone tests, drug of abuse tests, infections disease tests) and Application of EN & ISO Standards & Quality Management Systems (1997 – 2004). Consultant to medical companies in Shanghai & Hangzhou PRC in relation to the

Application of Quality Management Systems (2004). Consultant in nanotechnology (modification of surfaces on nano level: food industry, metal processing industry, glass industry) (2005).

3. I hold degrees of Bachelor of Science and Masters in Chemistry from Westf. Wilhelms Universitat Munster and DoctorNatrural Science (Dr. rer. nat.) from Technical University Darmstadt. Thesis: Conformational changes of tRNA during protein biosynthesis.

4. Some of my work in relation to the subject matter of this invention has been conducted in association with Professor Wolfgang Hillen of Friedrich-Alexander University, Erlangen, Germany. Professor Hillen is a world-renowned expert in the field of Microbiology. A copy of Professor Hillen's curriculum vitae is attached.

5. I have read and understood US Patent Application No. 10/039,677 (which I understand was published as US2003/0031687), the Office Action dated 12 July 2006 and the prior art documents to which the Examiner has referred. I am familiar with the amended claims currently under consideration.

6. I understand that the examiner considers that the claimed subject matter would have been obvious over Jackson (GB-A-2247171) in view of Dorothy (GB-A-2338651).

7. I have been responsible for conducting a number of experiments to illustrate the surprising and unexpected properties of the compositions of the invention. I have also studied experimental reports of other experiments that have been conducted to illustrate the advantages of the invention.

8. A summary of some of the experiments conducted and the results obtained is provided in attached Annexes I and II.

9. The attached Annexes are:

Annex I — Report of Experiments Conducted by Dr Schwarz and Prof Hillen to Illustrate the Characteristic Properties of the Compositions which are the subject of US Patent Application No. 10/039.677.

Annex II — Report of Experiments Conducted to Illustrate the Residual Effect of the Compositions which are the subject of US Patent Application No. 10/039,677.

10. Annex I describes an experiment that was conducted to illustrate the residual antimicrobial effect of compositions of the invention (see pages 5 to 7). The composition of the present invention was applied to a bathroom surface in two passenger cabins in a cruiser liner. The surface was then cleaned on a daily basis using water only. The total microbial count on the surface was tested on the third and the seventh day after application of the composition. The results reported show that the compositions of the invention provided a residual antimicrobial effect, largely reducing or controlling the formation of colonies of microorganisms on a surface for up to a week (the limit of this experiment) after application of the composition even when that surface is washed daily with water.

11. Annex II also describes some experiments that illustrate the residual antimicrobial effect of the compositions of the invention. A composition of the invention was applied to a surface and allowed to dry on that surface. When the surface was dry, a protein solution was applied to the surface and was dried. The surface was then rinsed extensively with distilled water. The water rinsing removed most of the protein solution from the surface, but did not remove the inventive composition from the surface. A comparative test was also carried out in which the same procedure was followed except that an anti-microbial composition that did not comprise a polysiloxane was used in place of the composition of the invention. In this comparative test rinsing extensively with distilled water did not remove the protein layer. These results indicate that a microbial biofilm would be unable to adhere to a surface treated with the composition of the present invention while it would be able to adhere to a surface treated with the comparative anti-microbial composition. This experiment illustrates the residual effect of the inventive composition in that in addition to killing microbes present at a surface at the time a surface is treated with the composition, further microbial biofilm formation at that surface is also prevented.

12. Annex I also describes a number of experiments that were conducted to illustrate the surprising and unexpected antimicrobial effect that is achieved by the combination of a quaternary ammonium compound and a low surface tension material, as defined in the claims of the present application, as compared to a quaternary ammonium compound in the absence of a low surface tension material (similar to Jackson).

13. In one of the experiments described in Annex I, cotton cloths were soaked in three compositions or deionised water (control). The three compositions were a solution of low surface tension material such as Clearco (a polysiloxane, similar to Dorothy), a solution of a quaternary ammonium compound and a solution of Clearco and the quaternary ammonium compound. The treated cloths were stored at room temperature until they were completely dry. Each of the dry pre-treated cloths and the control cloths were placed into Petri dishes filled with 5 mL of deionised water and let stand for 5 minutes. The washed cloths were then dried. Each dry, washed cloth was challenged with 300 µL E. Coli and allowed to completely dry at room temperature. When dry, the cloths were incubated at 37°C for 2 hours. Deionised water was then added to each Petri dish to elute surviving E. coli. The plates were evaluated for viable colony forming units.

14. As can be seen from the report, the low surface tension materials (which are similar to Dorothy) do not themselves have any antimicrobial activity. The quaternary ammonium compounds (which are similar to Jackson) were effective only at concentrations of 1.5% and 0.07%, but not at 0.025%. However, when a low surface tension material is used in combination with a quaternary ammonium compound, the antimicrobial properties of the resulting composition are unexpectedly enhanced. In fact, when the low surface tension material was used in combination with the quaternary ammonium compound as claimed in the present invention, the composition was effective as an anti-microbial agent at concentrations of 2.5%, 1.5%, 0.125%, 0.07%, 0.042%, and even 0.025%. These results were confirmed in a second experiment which tested the compositions over a broader concentration range.

15. The composition of the present invention was unexpectedly more effective as an anti-bacterial agent than the either of the test compositions. It is especially surprising that a low surface tension material, which does not have any antimicrobial effect alone, would enhance the antimicrobial effect of the quaternary ammonium compound.

16. The results of these experiments illustrate that both the residual effect of the compositions of the invention and the enhanced antimicrobial effect that is achieved by the combination of a quaternary ammonium compound and a low surface tension material were surprising and unexpected. One could not have predicted that the combination of a quaternary ammonium compound and a low surface tension material was much more effective than either composition alone. This is especially surprising based on the fact that the low surface tension material was wholly ineffective as an antimicrobial composition. Similarly, one could not have predicted that the combination of a quaternary ammonium compound and a low surface tension material would have a residual effect on the growth of microbial colonies.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present application or any patent issued thereon.

Signed: Ulrich W. Schwarz

This 08 day of January 2007

(2)



# ANNEX I

# REPORT OF EXPERIMENTS CONDUCTED BY DR SCHWARZ AND PROF HILLEN TO ILLUSTRATE THE CHARACTERISTIC PROPERTIES OF THE COMPOSITIONS WHICH ARE THE SUBJECT OF US PATENT APPLICATION NO. 10/039,677 ASSIGNED TO BYOTROL PLC

## MATERIALS

The following materials were used in the experiments that are described in this report.

### LB Medium (Agar Medium)

LB Medium was used as buffer and liquid nutrient broth. This medium contained:

- 10 g/l Tryptone
- 5 g/l Yeast extract
- 10 g/l Sodium chloride
- pH was adjusted to 7

LB Agar plates containing this medium were also used.

### Quaternary Ammonium Compound: BAC

A solution comprising:

| CAS No | Compound | Concentration |
|---|---|---|
| 61789-71-7 | Coco alkyl dimethylbenzyl ammonium chloride | 12.5 % |

Was used as an example of the first anti-microbial agent as defined in currently pending claim 1 of US Patent Application No. 10/039,677. This solution was an aqueous solution of the quaternary ammonium compound and was obtained from Thor Ltd. The solution was diluted with distilled water as necessary to give the concentration used in the experiments reported here in.

### Low Surface Tension Compounds

The following materials were used as representative of the at least one compound having a low surface tension as defined in currently pending claim 1 of US Patent Application No. 10/039,677.

**ACTIVE SILICONE**

| CAS No | Compound | Concentration |
|---|---|---|
| 63148-62-9 | Polydimethylsiloxane | 2.5 % |
| | As stabilizer: Ethoxylated Nonyl Phenol [9016-45-9] | |

**CLEARCO**

| CAS No | Compound | Concentration |
|---|---|---|
| 541-02-6 | Decamethylcyclopentasiloxane | 2.5 % |
| | As stabilizer: Ethanol 75 % | |

**PS034**

| CAS No | Compound | Concentration |
|---|---|---|
| 107460 | Hexamethyldisiloxane<br>As stabilizer: Ethanol 75 % | 2.5 % |

All of these materials were obtained from Clearco.

**Byotrol Formulation**

As an example of a formulation of the invention containing the components as defined in currently pending claim 1 of US Patent Application No. 10/039,677 and at least one additional anti-microbial agent the following formulation was used.

**A1616 PRODUCT (F4L Ready To Use)**

| CAS-No. | COMPOUND | Active Conc. |
|---|---|---|
| 61789-71-7 | coco alkyl dimethylbenzyl ammonium chloride | 0.07 % |
| 7173-51-7 | di-n-decyl dimethylammonium chloride | 0.07 % |
| 52-51-7 | bronopol (INN) | 0,05 % |
| 27083-27-8 | Polymeric Biguanide Hydrochloride | 0,03 % |
| 64-17-5 | ethanol | 0,13 % |
| 990001-58-01 | polydol | 0,015 % |
| | water | 99,64 % |

This formulation will be referred to herein after as "the Byotrol formulation".

**SUPERNOVA WIPES**

Disposable wipes impregnated with the A1616 product (F4L Ready To Use). These wipes were taken from a tub containing approximately 150 wipes and approximately 700 ml of the Byotrol solution.

**CADDYCARE**

Caddycare is one brand name for the Byotrol A1616 F4L formulation described above. In these experiments it was used in spray form.

## DEMONSTRATION OF RESIDUAL EFFECT

A test to demonstrate the residual antimicrobial effect of compositions of the invention was conducted in the bathroom of two passenger cabins of a cruise liner.

A bathroom was checked for total microbial counts prior to cleaning. This was done using a commercial surface test (Environcheck, Merck). Three different areas of the bathroom were checked as shown in Figure 1. The same areas of each bathroom were tested in each of the tests carried out in the rest of this experiment.

The results for the uncleaned bathroom are shown in Figure 1 (Cabin A).

The bathrooms were then cleaned using standard methods. By this we mean that they were cleaned with water and a disinfecting cleaner (containing didecyldimethyl ammonium chloride and ethoxylated nonyl phenol)

The bathrooms were checked again for total microbial counts. The results are in Figure 1 (Cabins B and C).

From Figure 1 it can be seen that cleaning with the disinfecting cleaner and water was ineffective in killing micro-organisms present on the surfaces tested.

The bathrooms were then treated by spraying with the Byotrol formulation.

Daily cleaning of the two bathrooms was then carried out using water only. The total microbial count was checked on the third day and on the seventh day. The results are shown Figure 2.

These results clearly show that the compositions of the invention provide a residual effect in that they prevent growth of micro-organisms at a surface that they have been used to treat even when that surface has been subjected to repeated washing.

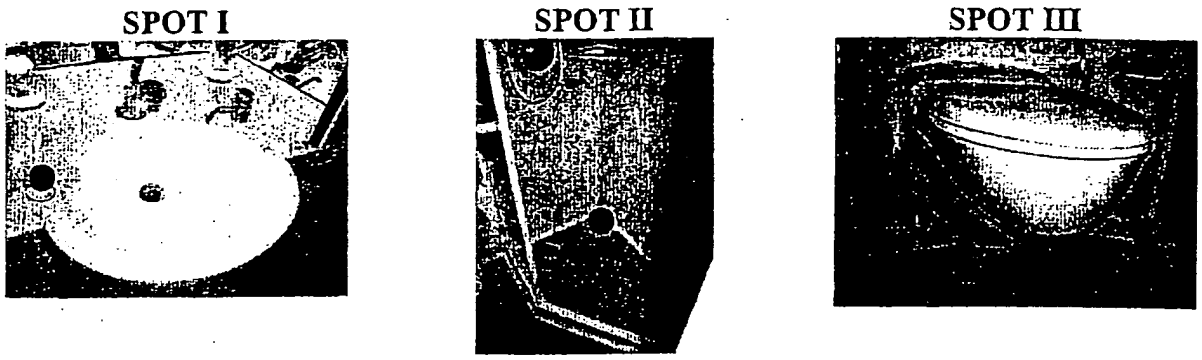
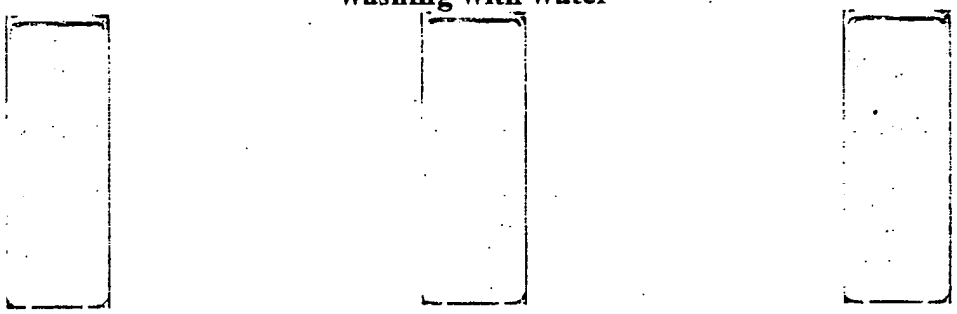

SPOT I
SPOT II
SPOT III
CABIN A - uncleaned
CABIN B – cleaned (with water and disinfectant cleaner)
CABIN C – cleaned (with water and disinfectant cleaner)

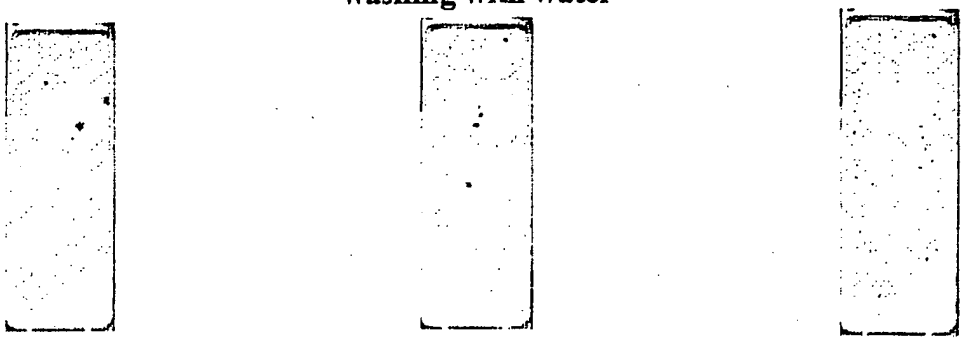
FIGURE 1

SPOT I SPOT II SPOT III

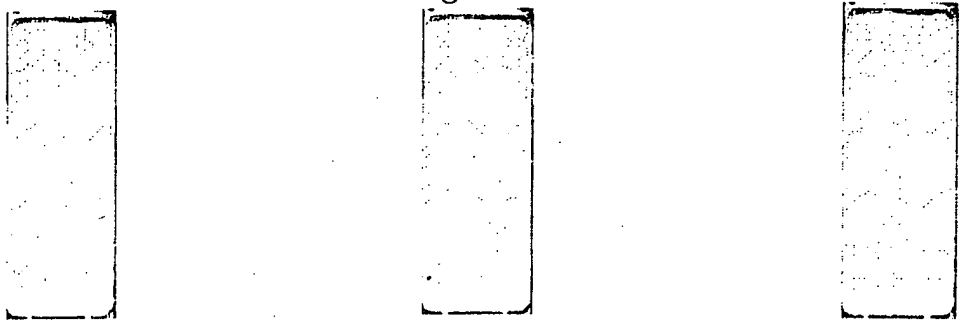
**CABIN B – 3 days after treatment with the A1616 F4L composition and after daily washing with water**



**CABIN B – 7 days after treatment with the A1616 F4L composition and after daily washing with water**



**CABIN C – 3 days after treatment with the A1616 F4L composition and after daily washing with water**



**CABIN C – 7 days after treatment with the A1616 F4L composition and after daily washing with water**

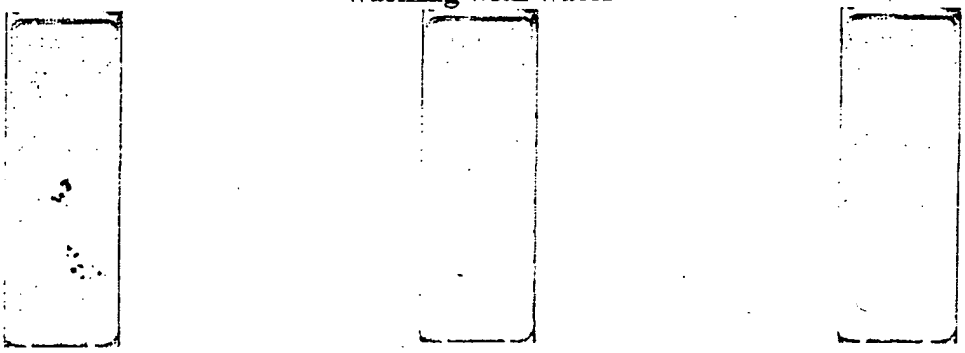


**FIGURE 2**

# TEST TO SHOW THE EFFECT OF THE COMBINATION OF A QUATERNARY AMMONIUM COMPOUND AND A LOW SURFACE TENSION MATERIAL

**Testing "additive effect" of polymer at limited biocide concentrations: Cloth Samples (1)**

Testing of "additive effect" was done at limited (very low) biocide concentrations.

## MATERIALS

The following materials were used:

Cloth (Cotton, 2x2 cm), LB Medium, BAC, CLEARCO, Wipe (Supernova soaked wipe), LB Agar plates, Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium ($OD_{600}$: 7.8 / mL (Reference: LB Medium) ≅ $7.8 \times 10^9$ cells/mL), deionised water.

**Test liquids**

| # | BAC | CLEARCO | BAC + CLEARCO | |
|---|---|---|---|---|
| | | | BAC | CLEARCO |
| 1 | 1.5 % | 2.5 % | 1.5 % | 2.5 % |
| 2 | 0.07 % | 0.125 % | 0.07 % | 0.125 % |
| 3 | 0.025 % | 0.042 % | 0.025 % | 0.042 % |

## TESTING PROCEDURE

**Pre-treatment of cloths**
The cloths were soaked with test liquids (as shown in the above table) or deionised water (control 1) and stored at room temperature till they were completely dry (about 1 hr). Control 2 (Supernova wipe) was also stored at room temperature.

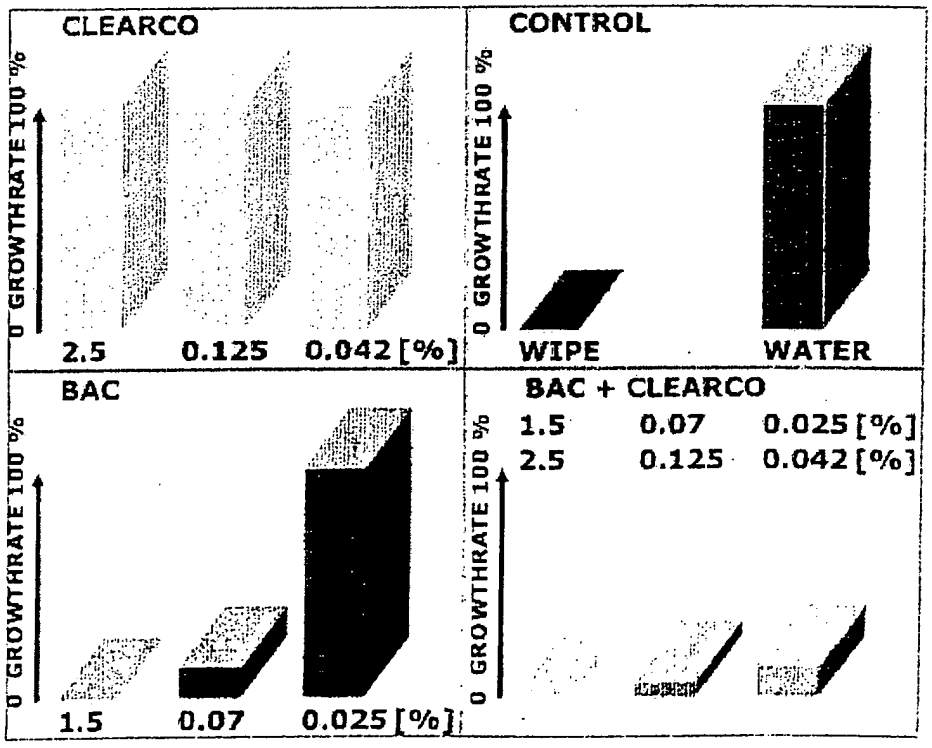
**Washing of pre-treated clothes**
Each of the dry pre-treated cloths and the control cloths were placed into a plastic Petri dish. The Petri dishes were filled with 5 mL of deionised water and left to stand for 5 minutes. The washed cloths were stored at room temperature till they were completely dry (about 1 hr).

**Challenge with E. coli**
Each dry pre-treated, washed cloth and each control cloths was challenged with 300 µL E. coli ($10^6$ cells in LB Medium) and allowed to completely dry at room temperature (about 30 minutes). When completely dry, the cloths were placed on LB Agar Petri dishes and incubated at 37 °C for 2 hrs. 200 µL deionised water was added to each Petri dish to elute surviving E. coli.

**Incubation**
The eluted E. coli samples were transferred to LB Agar plates and incubated at 37 °C for 48 hrs.

**Evaluation**
The plates were then evaluated for viable colony forming units (CFUs) such that 100% corresponds to an uncountable number of CFUs and 10% corresponds to approximately $10^2$ CFUs. The results are presented graphically below.

**Results and Interpretation**
As shown in the graphs below, under the test conditions described above BAC alone was active as an anti-microbial agent at concentrations of 1.5 % and 0.07 % but it was ineffective at a concentration of 0.025 %. The polymer CLEARCO showed no antimicrobial activity when used alone. The combination of BAC and CLEARCO showed strong antimicrobial activity at all the concentrations tested. Particularly significant is the fact that the combination of BAC and Clearco having a BAC concentration of 0.025% had much stronger antimicrobial activity that this concentration of BAC in the absence of the low surface tension material Clearco. The general activity of the test design was cross checked by using water as control 1 (no antimicrobial activity) and the Supernova Wipe (strong antimicrobial activity) as control 2.



**Testing "additive effect" of polymer at limited biocide concentrations: Cloth Samples (2)**

A test similar to that described above was carried out using BAC and CLEARCO over a large concentration range. Again, testing of "additive effect" was done at limited (very low) biocide concentrations (including concentrations lower than those used in the test described above).

## MATERIALS

Cloth (Cotton, 2x2 cm), LB Medium, BAC, CLEARCO, LB Agar plates, Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium ($OD_{600}$: 3.0 / mL (Reference: LB Medium) ≈ 3.0 x $10^9$ cells/mL), deionised water.

### Test liquids

| # | BAC | CLEARCO | BAC + CLEARCO | |
|---|---|---|---|---|
| | | | BAC | CLEARCO |
| 1 | 1.5 % | 2.5 % | 1.5 % | 2.5 % |
| 2 | 0.07 % | 0.125 % | 0.07 % | 0.125 % |
| 3 | 0.051 % | 0.098 % | 0.051 % | 0.098 % |
| 4 | 0.038 % | 0.064 % | 0.038 % | 0.064 % |
| 5 | 0.031 % | 0.051 % | 0.031 % | 0.051 % |
| 6 | 0.025 % | 0.042 % | 0.025 % | 0.042 % |
| 7 | 0.021 % | 0.036 % | 0.021 % | 0.036 % |
| 8 | 0.019 % | 0.031 % | 0.019 % | 0.031 % |
| 9 | 0.017 % | 0.028 % | 0.017 % | 0.028 % |
| 10 | 0.015 % | 0.025 % | 0.015 % | 0.025 % |
| 11 | 0.013 % | 0.023 % | 0.013 % | 0.023 % |

## TESTING PROCEDURES

### Pre-treatment of cloths

The cloths were soaked with test liquids (as shown in the above table) or deionised water (control 1) and stored at room temperature till they were completely dry (about 1 hr).

### Washing of pre-treated clothes

Each of the dry pre-treated cloths and the control cloths were placed into a plastic Petri dish. The Petri dishes were filled with 5 mL of deionised water and left to stand for 5 minutes. The washed cloths were stored at room temperature till they were completely dry (about 1 hr).

**Challenge with E. coli**

Each dry pre-treated, washed cloth and each control cloths was challenged with 100 µL E. coli (3 x $10^4$ cells in LB Medium) and allowed to completely dry at room temperature (about 30 minutes). When completely dry, the cloths were placed in 1 mL LB Medium for extraction of E. coli (about 10 minutes). 100 µL of extraction buffer were diluted with 900 µL LB Medium. 300 µL of this dilution were placed on Agar Plates. 300 µL should contain ~ $10^3$ E. coli.

**Incubation**

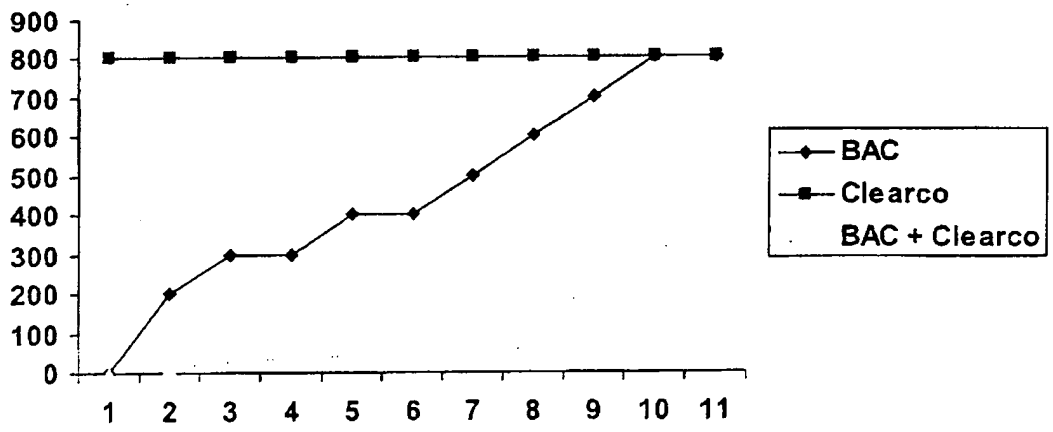
The E. coli samples were incubated at 37 °C for 48 hrs.

**Evaluation**

A grid method was used to count the number of CFUs in a given area. The results are presented graphically below (where the numbers on the X axis represent the test liquid number in the Table above and the numbers on the Y axis represent the number of CFUs in a square of the grid).

**Results and Interpretation**

As shown in the graph below, under the test conditions described above BAC alone was active as an anti-microbial agent at the higher concentrations used but as the concentration decreased BAC alone became rapidly less effective and it was ineffective at lower concentrations. The polymer CLEARCO showed no antimicrobial activity when used alone. The combination of BAC and CLEARCO showed strong antimicrobial activity at even at lower concentrations of BAC. The general activity of the test design was cross checked by using water as control 1 (no antimicrobial activity).



**Testing "additive effect" of polymer at limited biocide concentrations: Pretreatment Of Agar Plates**

Testing of "additive effect" was done at limited (very low) biocide concentrations.

## MATERIALS

LB Agar plates, LB Medium, BAC, CLEARCO, Caddycare (contains A1616 F4L Ready To Use), Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium ($OD_{600}$: 4.3 / mL (Reference: LB Medium) ≈ 4.3 x $10^9$ cells/mL), deionised water.

### Test liquids

| # | BAC | CLEARCO | BAC + CLEARCO | |
|---|---|---|---|---|
| | | | BAC | CLEARCO |
| 1 | 1.5 % | 2.5 % | 1.5 % | 2.5 % |
| 2 | 0.07 % | 0.125 % | 0.07 % | 0.125 % |
| 3 | 0.025 % | 0.042 % | 0.025 % | 0.042 % |

## TESTING PROCEDURES

### Pre-treatment of agar plates

1 mL of the test solutions (as shown in the above Table) was added to LB Agar plates and spread of the surface of the plates as a film. As control 1, 1 ml of deionised water was added to a LB Agar plate and spread of the surface of the plate as a film. As control 2, 1 ml of Caddycare was added to a LB Agar plate and spread of the surface of the plate as a film. The treated LB Agar plates were stored at room temperature for 6 hrs to allow the test solutions to diffuse into the agar.

### Challenge with E. coli

Each pre-treated LB Agar plate was challenged with 300 µL E. coli ($10^6$ cells in LB Medium) covering the agar surface.

### Incubation

The Agar plates were incubated at 37 °C for 48 hrs.

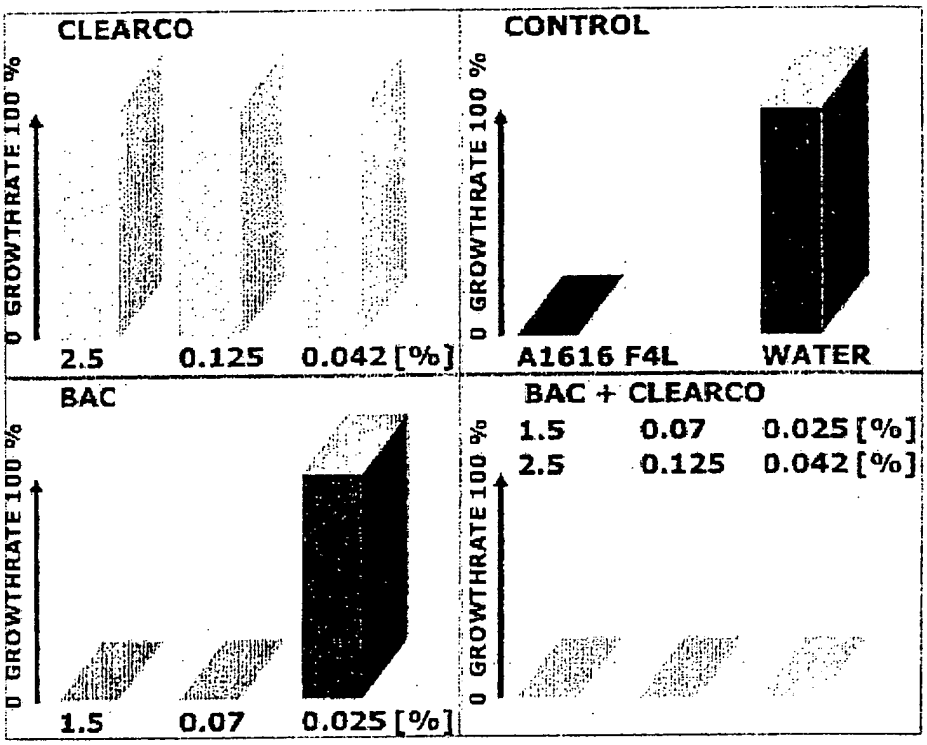
### Evaluation

The plates were then evaluated for viable colony forming units (CFUs) such that 100% corresponds to an uncountable number of CFUs and 10% corresponds to approximately $10^2$ CFUs. The results are presented graphically below.

### Results and Interpretation

As shown in the graphs below, under the test conditions described above BAC alone was active as an anti-microbial agent at concentrations of 1.5 % and 0.07 % but it was ineffective at a concentration of 0.025 %. The polymer CLEARCO showed no antimicrobial activity when used alone. The combination of BAC and CLEARCO

showed strong antimicrobial activity at all the concentrations tested. Particularly significant is the fact that the combination of BAC and Clearco having a BAC concentration of 0.025% had much stronger antimicrobial activity that this concentration of BAC in the absence of the low surface tension material Clearco. The general activity of the test design was cross checked by using water as control 1 (no antimicrobial activity) and the Caddycare (strong antimicrobial activity) as control 2.



**Testing "additive effect" of different polymers at limited biocide concentrations: Pretreatment Of Agar Plates**

Testing of "additive effect" was done at limited (very low) biocide concentrations and combinations of the biocide (BAC) and the low surface tension materials CLEARCO, PS034, and ACTIVE SILICONE were used.

**MATERIALS**

LB Agar plates, LB Medium, BAC, CLEARCO, PS034, and ACTIVE SILICONE, Eppendorf pipette tips, Eppendorf tubes (1 mL), E. coli ATCC 1036 overnight culture in LB Medium ($OD_{600}$: 4.3 / mL (Reference: LB Medium) ≙ 4.3 x $10^9$ cells/mL), deionised water.

**Test liquids**

| BAC | CLEARCO | PS034 | SILICONE |
|---|---|---|---|
| 0 % | | | |
| 0.019 % | 0.031 % plus 0.93 % Ethanol | 0.031 % plus 0.93 % Ethanol | 0.031 % |
| 0.015 % | | | |
| 0.0125 % | | | |
| 0.011 % | | | |

Compositions comprising each of the polymers and BAC at each of the concentrations shown above were prepared.

**TESTING PROCEDURES**

**Pre-treatment of agar plates**
1 mL of the test solutions (as shown above) was added to LB Agar plates and spread of the surface of the plates as a film.

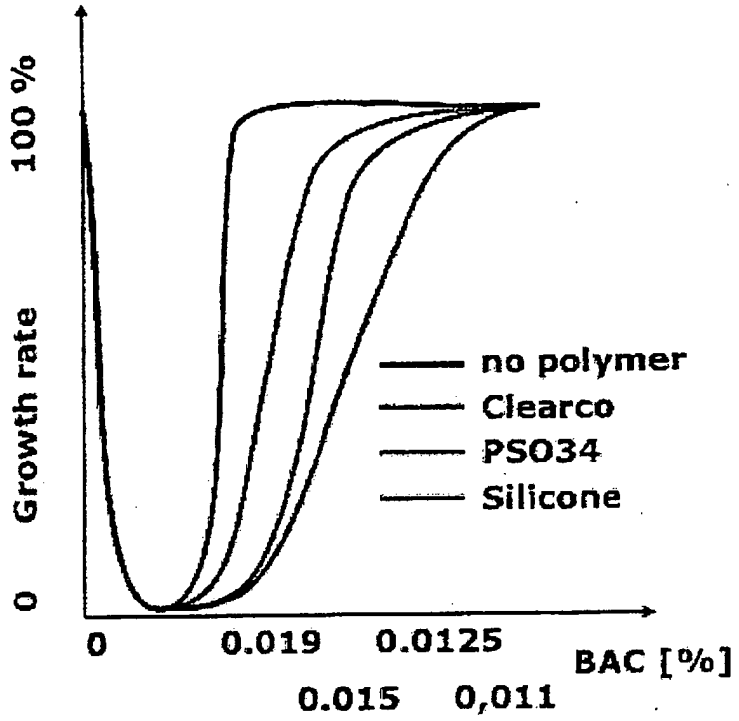
**Challenge with E. coli**
Each pre-treated LB Agar plate was challenged with 300 µL E. coli ($10^6$ cells in LB Medium) covering the agar surface.

**Incubation**
The Agar plates were incubated at 37 °C for 48 hrs.

**Evaluation**
The plates were then evaluated for viable colony forming units (CFUs) such that 100% corresponds to an uncountable number of CFUs and 10% corresponds to approximately $10^2$ CFUs. The results are presented graphically below.

**Results and Interpretation**

As shown in the graph below, all of the polymers used significantly enhanced the efficacy of the BAC in compositions containing a low concentration of BAC compared to the same concentration of BAC in the absence of a polymer.



(3)

# ANNEX II

## REPORT OF EXPERIMENTS CONDUCTED TO ILLUSTRATE THE RESIDUAL EFFECT OF THE COMPOSITIONS WHICH ARE THE SUBJECT OF US PATENT APPLICATION NO. 10/039,677

Experiments were conducted to show the residual effect of the compositions that are the subject of US patent application no. 10/039677.

### EXPERIMENT SET I

The antimicrobial formulation Marquat MQ624M was used. This formulation comprises:

| ACTIVE INGREDIENTS: | |
|---|---|
| Octyl Decyl Dimethyl Ammonium Chloride | 3.0% |
| Didecyl Dimethyl Ammonium Chloride | 1.5% |
| Dioctyl Dimethyl Ammonium Chloride | 1.5% |
| Alkyl ($C_{14}$, 50%; $C_{12}$, 40%; $C_{16}$, 10%) dimethyl benzyl ammonium chloride | 4.0% |
| **INERT INGREDIENTS:** | 90.0% |
| **TOTAL** | 100.0% |

This material was used neat and was also used in mixture with 1% and 5 % by weight of the Marquat MQ624M of a polydimethylsiloxane polymer (obtained from Fluorochem as Fluorochem 034).

As used hereinafter, the neat formulation (Marquat MQ624M) will be referred to as formulation A, the formulation comprising 1 % by weight of the polysiloxane will be referred to as formulation B and the formulation comprising 5 % by weight of the polysiloxane will be referred to as formulation C.

Formulation A is a control in that it does not contain the polymer, which is an essential feature of the compositions of the invention which is the subject of US Patent Application No. 10/039677. Formulations B and C are illustrative examples of compositions of the invention containing different concentrations of the polymer.

### Experiment 1

A polyethylene Petri-dish was treated with 1 mL of formulation A and the surface was allowed to dry. When the surface was completely dry 1 mL of protein solution (about 3 % casein peptone mixture in liquid broth) was applied. The applied mixture was

dried at about 20 °C for at least one hour. This dried material was used to simulate the *extra*-cellular secretions of certain bacteria that constitute the "adherant" part of the biofilm formed by those bacteria at a surface.

The surface was then rinsed extensively with distilled water.

The rinsing step did not remove the protein layer or the layer of formulation A.

**Experiment 2**

The procedure of Experiment 1 was repeated except that 1 mL of formulation B was used instead of formulation A.

The rinsing step removed most of the protein layer but did not remove the layer of formulation B.

**Experiment 3**

The procedure of Experiment 1 was repeated except that 1 mL of formulation C was used instead of formulation A.

The rinsing step removed nearly all of the protein layer but did not remove the layer of formulation C.

**Comments**

As described in Experiment 1 above, the protein layer used in each of experiments 1 to 3 was considered to simulate the biofilm of a microbial colony.

The results obtained in Experiment 1 indicate that a biofilm would be able to adhere is a surface treated with the antimicrobial formulation A (ie neat Marquat MQ624M). The results obtained in Experiments 2 and 3 indicate that a biofilm would be unable to adhere to a surface treated with an anti-microbial composition of the invention containing a polydimethylsiloxane (formulations B and C).

As indicated at page 1, lines 22 and 23 of the application, adhesion to a surface is an essential step in biofilm formation. Thus, the results obtained show that the presence of a polydimethylsiloxane in an antimicrobial composition will significantly reduce the ability of a microbial colony to form on a treated surface. This results in a residual effect, ie not only do the compositions of the invention kill microbes present at a surface at the time a surface is treated with the composition, they also prevent further biofilm formation at the surface to which they have been applied. This effect could not have been predicted from either of the documents cited by the examiner.

Further experiments were undertaken to demonstrate that the compositions of the invention continue to exhibit anti-microbial properties at a surface to which they have been applied in addition to suppressing biofilm formation as illustrated above.

**EXPERIMENT SET II**

**Experiment 4**

500 µL of E. coli solution that approximated to $10^5$ colony forming units was applied to each of 6 HDPE (High Density Polyethylene) Petri dishes which had each been treated with different concentrations of the following formulation (F4L) as indicated in the Table below:

***F4L***

| ***Composition*** | ***%*** |
|---|---|
| Coco alkyl dimethylbenzyl ammonium chloride | 20.000% |
| di-n-decyl dimethylammonium chloride | 20.000% |
| bronopol (INN) | 20.000% |
| Polymeric Biguanide Hydrochloride | 20.000% |
| water | 0.000% |
| ethanol | 18.000% |
| Poly-dimethyl siloxane | 2.000% |
| **Totals:** | 100.00% |

Formulation F4L is a composition of the invention as defined in patent application no. 10/039677.

| **Petri-dish** | | **Concentration of F4L** |
|---|---|---|
| A | Control | Distilled water only |
| B | Neat | No added water (100% Conc) |
| C | 1:4 | 1 part F4L: 4 parts Water (20% Conc) |
| D | 1:9 | 1 part F4L : 9 parts water (10% Conc) |
| E | 1:19 | 1 part F4L : 19 Parts water (5% Conc) |
| F | 1:39 | 1 part F4L : 39 parts water (2.5% Conc) |
| G | 1:79 | 1 part F4L : 79 parts water (1.25% Conc) |

15 minutes after the applied solution had completely dried 500 µL of liquid broth were used to dissolve E. coli. From each of the Petri dishes. 300 µL of the solution obtained from each Petri dish was applied to a separate LB agar plate. The LB agar plates were then incubated for 24 hrs at 37 °C. Standard methods were then used to determine the presence of E. coli on the LB agar plates.

There were no visible colonies present on any of the LB agar plates obtained from Petri dishes, which had been treated with the F4L formulation at any dilution. However, on the LB plate obtained from the control Petri dish (A) the number of E. coli colonies was too numerous to count.

**Comments**

The results of this experiment show that the compositions of the invention when present at a surface kill microbes that contact that surface subsequent to application of a composition of the invention to the surface even at dilutions as much as 1:79 (F4L:deionizeed water).

**Conclusions**

The experiments reported above illustrate that the compositions that are the subject of US patent application no. 10/039677 have a residual effect in that they prevent biofilm formation at a surface to which they have been applied and they also kill micorobes that contact such a surface subsequent to that surface being treated with a composition of the invention.

C

## Related Proceedings Appendix

None.